

Ethnobotany, leaf anatomy, essential oil composition and antibacterial activity of *Pteronia onobromoides* (Asteraceae)

I.M. Hulley^a, A.M. Viljoen^b, P.M. Tilney^a, S.F. Van Vuuren^c, G.P.P. Kamatou^b, B.-E. Van Wyk^{a,*}

^a Department of Botany and Plant Biotechnology, University of Johannesburg, P.O. Box 524, Auckland Park 2006, Johannesburg, South Africa

^b Department of Pharmaceutical Sciences, Tshwane University of Technology, Private Bag X680, Pretoria 0001, South Africa

^c Department of Pharmacy and Pharmacology, Faculty of Health Sciences, University of the Witwatersrand, 7 York Road, Parktown 2193, Johannesburg, South Africa

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Abstract

Available ethnobotanical information on *Pteronia onobromoides* (first recorded in 1685) indicates that the plant was once of considerable cultural and commercial importance and that it was powdered, mixed with fat, and applied to the skin for cosmetic and/or medicinal purposes. *Sáb*, as well as *Son* or *San*, are considered to be the original Nama names for this aromatic bush and also the origin of various names for San people, such as *Sonqua* and *Bushman*. A study of the leaf anatomy showed that essential oil is produced in globose oil glands situated below some of the vascular bundles in the spongy parenchyma, adjacent to the palisade parenchyma. The oil is relatively complex but contains a combination of myrcene, limonene, 1,8-cineole and *p*-cymene as main compounds, with smaller amounts of sabinene, *trans*-linalooloxide, linalool, terpinen-4-ol, α -terpineol, eugenol, thymol and α -phellandrene. Dichloromethane extracts exhibited antibacterial activity (especially against *Staphylococcus epidermidis*) with MIC values as low as 0.83 mg/ml. Other solvent extracts and the essential oil itself were less active. The results show that the traditional method of mixing powdered leaves with fat and applying it to the skin may have had deodorant, disinfectant and medicinal benefits. © 2009 SAAB. Published by Elsevier B.V. All rights reserved.

Keywords: Asteraceae; Buchu; Essential oil; Ethnobotany; Leaf anatomy; MIC values; *Pteronia onobromoides*

1. Introduction

Pteronia onobromoides DC. (Asteraceae) is one of a genus of ca. 70 species of woody shrublets subendemic to southern Africa (Leistner, 2000). The plant has a localized distribution and is restricted to the coastal sand dunes along the west coast of South Africa, from near Cape Town to the vicinity of Springbok in Namaqualand. It is a much-branched, leafy shrub (Fig. 1A) with glabrous, smooth white stems bearing broadly linear leaves with prominent, acute ivory white teeth on the strongly incurved margins (Fig. 1B). The large, single flower heads comprise several bright yellow disc florets (ray florets are absent), surrounded by a few rows of glabrous, overlapping involucre bracts (Fig. 1B). The flowering time is from September to December.

The species has an interesting but poorly recorded ethnobotanical history as one of the main sources of aromatic leaf powders

(known as “buchu”), used specifically by the Nama people. Despite the obvious ethnobotanical importance of the species, it has remained scientifically poorly known and only a few anecdotes are known. No published details are available on the leaf anatomy, the oil glands and the volatile constituents of the species.

The aims of this paper are to provide a summary and synthesis of all known ethnobotanical information on *P. onobromoides*, to study the leaf anatomy with special emphasis on the structure of the oil glands and to describe, for the first time, the chemical composition of the essential oil of the species. In view of the traditional importance of the plant in skin care, we also investigated possible antibacterial activity.

2. Material and methods

2.1. Materials studied

Fresh material of *P. onobromoides* was collected at three localities along the west coast of South Africa, namely

* Corresponding author.

E-mail address: bevanwyk@uj.ac.za (B.-E. Van Wyk).

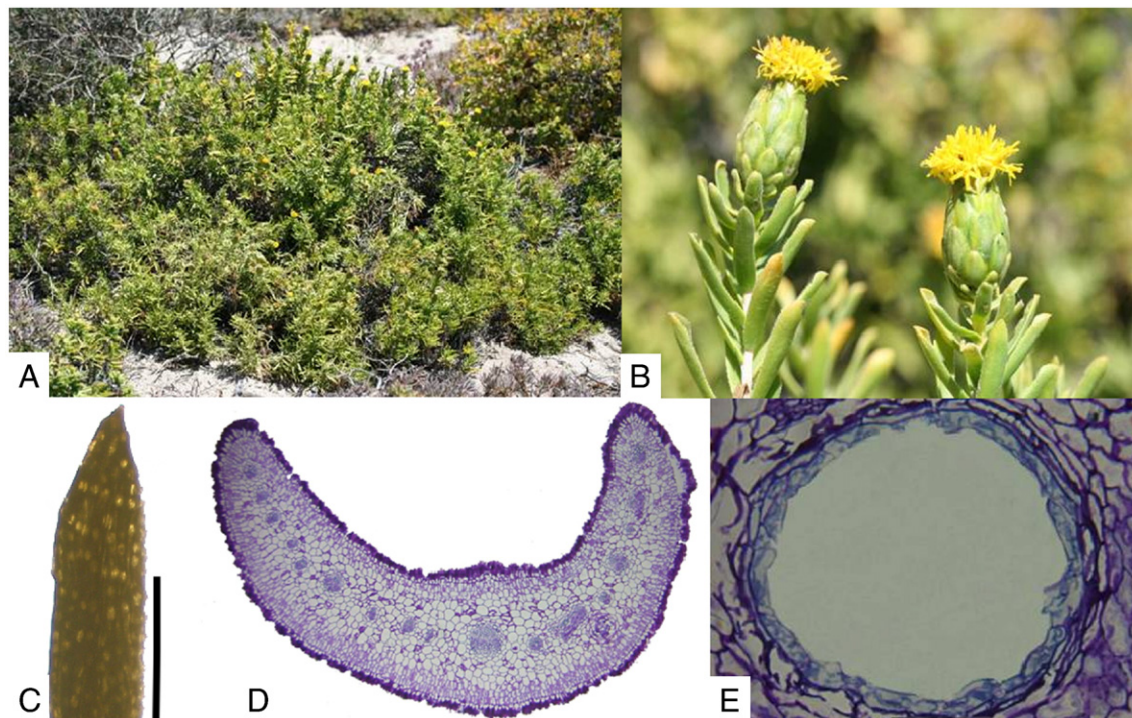


Fig. 1. *Pteronia onobromoides*: habit (A); leaves and flower heads (B); leaf showing oil glands (visible as translucent dots) (C); transverse section of leaf showing an oil gland (D); single oil gland showing cellular details (E). Scale bars: C=4 mm, D=0.4 mm, E=0.07 mm.

Dwarskersbos [32° 18' CA], Nortier Experimental Farm, north of Lamberts Bay [32° 18' AB] and 2 km south of Lamberts Bay [32° 18' AB]. The samples were air dried. Exact localities and voucher specimen details are presented in Table 1.

2.2. Anatomical procedures

Dried leaf material was rehydrated and then placed in formalin–acetic acid–alcohol (FAA; 5:5:95) for 24 h. Small portions of the leaves were cut and then treated according to the glycol methacrylate (GMA) method of Feder and O'Brien (1968). This involves dehydrating the material through a graded alcohol series before infiltrating with GMA and embedding in capsules containing GMA. The capsules were placed in an oven at 60 °C for 24 h to polymerize. Sections, 3–5 µm thick, were made using an ultramicrotome. Staining was done with Schiff's reagent and toluidine blue. The microscope slides were observed under a light microscope equipped with a digital camera and a computerised data capturing system.

2.3. Distillation and analysis of essential oil

Air-dried leaves of six samples (Table 1) were subjected to hydrodistillation for 180 min using a Clevenger-type apparatus. The oils were weighed and stored in sealed vials in the dark, at 4 °C, before analysis.

The oils (20% diluted in hexane) were analysed by gas chromatography–mass spectrometry (GC–MS, Agilent 6890N GC system coupled directly to a 5973 MS). A volume of 1 µl was injected (using a split ratio of 200:1) with an autosampler at

24.79 psi and an inlet temperature of 250 °C. The GC system equipped with a HP-Innowax polyethylene glycol column (60 m × 250 µm i.d. × 0.25 µm film thickness) was used. The

Table 1
Voucher specimen details of the plant materials of *P. onobromoides* that were studied.

Sample number	Locality	Voucher specimens (all housed in JRAU)	Anatomy (A) extracts for MIC studies (MIC) GC-MS
1	Nortier Experimental Farm (NEF), Lambert's Bay (close to sea)	<i>Rheeder s.n. sub Van Wyk 4246a</i>	A, MIC, GC-MS
2	NEF, Lambert's Bay (100 m from sea)	<i>Rheeder s.n. sub Van Wyk 4246b</i>	MIC,
3	NEF, Lambert's Bay (200 m from sea)	<i>Rheeder s.n. sub Van Wyk 4246c</i>	MIC, GC-MS
4	NEF, Lambert's Bay (300 m from sea)	<i>Rheeder s.n. sub Van Wyk 4246d</i>	MIC, GC-MS
5	2 km S of Lambert's Bay (close to sea)	<i>B & M Van Wyk 4273a</i>	MIC
6	2 km S of Lambert's Bay (close to sea)	<i>B & M Van Wyk 4273b</i>	MIC
7	2 km S of Lambert's Bay (close to sea)	<i>B & M Van Wyk 4273c</i>	MIC
8	Dwarskersbos (close to sea)	<i>B & M Van Wyk 4311a</i>	MIC, GC-MS
9	Dwarskersbos (close to sea)	<i>B & M Van Wyk 4311b</i>	MIC, GC-MS
10	Dwarskersbos (close to sea)	<i>B & M Van Wyk 4311c</i>	MIC, GC-MS

The samples were taken from single plants at three localities.

oven temperature program was 60 °C for the first 10 min, rising to 220 °C at a rate of 4 °C/min and held for 10 min and then rising to 240 °C at a rate of 1 °C/min. Helium was used as carrier gas at a constant flow of 1.2 ml/min. Spectra were obtained on electron impact at 70 eV, scanning from 35 to 550 m/z. The percentage compositions of the individual components were obtained from electronic integration measurements using flame ionization detection (FID, 250 °C). *n*-Alkanes were used as reference points in the calculation of relative retention indices (RRI). Component identifications (Table 2) were made by comparing mass spectra and retention indices. Library searches were carried out using NIST[®], Mass Finder[®] and Flavour[®] libraries.

2.4. Antibacterial studies

Oil samples and various extracts (Table 3) were investigated for antimicrobial activity using the minimum inhibitory concentration (MIC) microtitre plate method described by Eloff (1998). Extracts were prepared by mixing 1 g of powdered leaf with 25 ml of solvent and leaving it overnight. The solvents used were a 1:1 mixture of methanol and water, a 1:1 mixture of

methanol and dichloromethane and sterilized, deionised water. These were filtered and dried in a fume hood (organic solvents) or freeze-dried (aqueous solvents). All MIC assays were undertaken in triplicate. Two Gram-positive bacterial strains (*Staphylococcus aureus* ATCC 6538 and *Staphylococcus epidermidis* ATCC 2223) and two Gram-negative bacterial strains (*Pseudomonas aeruginosa* ATCC 27858 and *Proteus vulgaris* ATCC 33420) were selected on the basis that these pathogens are commonly associated with skin and wound infections. Bacterial cultures were subcultured from stock agar plates and grown in Tryptone Soya broth for 18 h. Extracts or oils diluted in acetone were applied (100 µl) to the first row of the microtitre plate at starting concentrations of 32 mg/ml (extracts) and 64 mg/ml (essential oils). Serial doubling dilutions were performed to yield concentrations varying from 32 mg/ml to 0.5 mg/ml. The cultures were diluted to an approximate inoculum size of 1×10^8 colony forming units (CFU)/ml and then introduced to all wells of the microtitre plate. Ciprofloxacin at starting stock concentrations of 0.01 mg/ml were used as positive controls against all test pathogens. The microtitre plates were sealed with sterile adhesive and incubated for 24 h at 37 °C. The colour reagent *p*-iodonitrotetrazolium violet (INT) was prepared (0.4 mg/ml) and 40 µl was transferred to all the inoculated wells after incubation. The microtitre plates were examined for colour changes (indicating microbial growth) after 6 h. The MIC value was read as the lowest dilution having no evidence of bacterial growth.

Table 2

The main compounds (percentage area) of essential oil samples from a selection of six individual plants of *Pteronia onobromoides* from two localities, as identified by GC-MS.

RRI	Major compounds/yield	Lamberts Bay			Dwarskersbos		
		S1	S3	S4	S8	S9	S10
		0.0025	0.5	0.18	0.66	0.38	0.0027
1017	α-Pinene	0.7	1.1	Tr	0.2	0.4	0.4
1020	α-Thujene	–	0.7	Tr	–	tr	0.5
1026	3-Buten-2-ol-2-methyl	–	1.0	0.1	0.6	0.2	0.1
1103	β-Pinene	1.8	2.0	0.1	0.7	0.9	0.9
1117	Sabinene	3.3	14.0	0.2	2.0	2.7	9.4
1160	Myrcene	42.9	16.6	1.2	29.5	44.6	7.0
1162	α-Phellandrene	6.2	–	–	–	6.2	1.0
1174	α-Terpinene	–	0.9	0.1	0.2	0.1	0.9
1193	Limonene	1.3	1.2	51.1	25.2	1.3	16.2
1203	1,8-Cineole	9.4	7.0	1.7	9.4	9.4	2.5
1241	γ-Terpinene	0.09	1.5	0.2	0.4	0.3	1.7
1267	p-Cymene	11.0	20.2	10.3	4.5	6.7	12.3
1437	<i>trans</i> -Linalooloxide	1.0	1.8	2.3	3.5	4.3	2.9
1460	<i>trans</i> -Sabinene-hydrate-acetate	0.5	3.0	0.1	0.2	0.6	2.0
1467	<i>cis</i> -Linalooloxide	0.4	0.8	1.1	1.8	2.3	1.4
1542	Linalool	5.4	1.4	4.3	3.1	0.9	15.7
1544	<i>trans-p</i> -Menth-2-en-1-ol	–	3.2	–	–	0.5	2.4
1595	Terpinen-4-ol	0.4	6.9	0.4	1.8	1.0	7.0
1692	α-Terpineol	1.3	2.2	0.6	6.7	3.0	1.2
1721	α-Farnesene	0.9	0.2	0.1	0.2	0.5	0.2
1799	<i>cis</i> -Sabinol	0.3	1.4	0.8	0.4	0.8	0.8
2008	Caryophyllene-oxide	0.1	1.0	3.7	–	0.1	0.4
2030	Methyleugenol	1.3	–	0.5	0.2	0.4	0.2
2186	Eugenol	1.2	0.2	3.2	2.4	1.6	2.0
2198	Thymol	3.0	0.3	0.3	–	4.0	1.3
2239	Carvacrol	0.2	0.2	0.6	0.3	0.3	0.4
	Total	92.7	88.8	83.0	93.3	93.1	90.8

tr: trace amount (<0.05%).

Sample numbers of the individual plants studied are given as in Table 1. Yield figures are in % w/w. Compound listed in bold are the major compounds in most of the samples.

3. Results and discussion

3.1. Ethnobotanical review

The earliest record of *P. onobromoides* can be found in the diary of Simon Van der Stel's journey to Namaqualand in 1685 (De Wet and Pheiffer, 1979). The species is clearly recognizable as Plate 791, recorded as having been collected on the 14th of September 1685 (Fig. 2). Unfortunately, no other details are provided about the species or its uses. Since practically all the plants illustrated by Hendrik Claudius (who went along on the expedition for this purpose) are accompanied by notes on their Nama names and local uses (mainly as food or medicine), it is very likely that *P. onobromoides* was illustrated because of its practical importance to the Namaqua. This assumption is confirmed by the only two other original anecdotes from Namaqualand (see later on).

Despite this early record in 1685, the ethnobotanical history of *P. onobromoides* has remained poorly known. Pappe (1847, 1850, 1857) did not include the species in his early publications of Cape medicinal plants. The first explicit record of cosmetic and medicinal uses by the Nama people dates back to 1854. William Guybon Atherstone (1814–98), a well-known medical doctor and naturalist, spent 5 months in Namaqualand in 1854–55. He was quoted by Harvey and Sonder (1865): “the leaves are succulent and very aromatic, used by the native Namaquas and Bastards as a perfume, mixed with fat, under the name buchu. It is called *Sāb* in the Namaqua language, and is dried and collected for sale”. This information was directly cited by Hutchinson and Phillips (1917)

Table 3
Minimum inhibitory concentrations (MIC's) for various extracts and essential oils of *Pteronia onobromoides*.

Extracts/samples used	Sample number (as in Table 1)	MIC (mg/ml)			
		<i>Staphylococcus aureus</i> ATCC 6538	<i>Staphylococcus epidermidis</i> ATCC 2223	<i>Pseudomonas aeruginosa</i> ATCC 27858	<i>Proteus vulgaris</i> ATCC 33420
H ₂ O extract	1	≥ 8	≥ 8	≥ 8	≥ 8
H ₂ O extract	4	8.00	≥ 8	8.00	≥ 8
MeOH:H ₂ O extract	1	≥ 8	≥ 8	8.00	≥ 8
MeOH:H ₂ O extract	2	≥ 8	8.00	8.00	8.00
MeOH:H ₂ O extract	3	≥ 8	6.00	8.00	8.00
MeOH:H ₂ O extract	4	≥ 8	8.00	≥ 8	8.00
MeOH:H ₂ O extract	5	≥ 8	8.00	≥ 8	≥ 8
MeOH:H ₂ O extract	6	≥ 8	6.00	≥ 8	≥ 8
MeOH:H ₂ O extract	7	8.00	2.00	≥ 8	≥ 8
MeOH:H ₂ O extract	8	≥ 8	6.00	≥ 8	≥ 8
MeOH:H ₂ O extract	9	≥ 8	5.33	≥ 8	≥ 8
MeOH:H ₂ O extract	10	≥ 8	8.00	≥ 8	≥ 8
MeOH:CH ₂ Cl ₂ extract	1	1.00	0.83	2.00	1.00
MeOH:CH ₂ Cl ₂ extract	4	2.00	1.67	2.00	1.00
Essential oil	1	6.00	4.00	2.00	3.00
Essential oil	3	4.00	4.00	3.00	2.00
Essential oil	4	6.00	5.6	3.00	1.00
Negative control (H ₂ O)		≥ 8	≥ 8	≥ 8	≥ 8
Negative control (C ₃ H ₆ O)		≥ 16	16	≥ 16	≥ 16
Positive control (ciprofloxacin)		0.156 µg	0.030 µg	0.313 µg	0.156 µg

without adding anything new. It is interesting that the Nama name “Sab” was also reported by Laidler (1928) as a synonym for buchu, who ascribed this record to Tyndale.

Marloth (1932) briefly recorded the species and its uses: “... and possesses a powerful odour not pleasant to the botanist, hence the specific name of the shrub, but much appreciated by the natives of Namaqualand as a medicine and cosmetic, and employed instead of buchu...” The only information given by Watt and Breyer-Brandwijk (1962) is cited directly from Marloth. Von Reis Altschul (1973) listed the same information, taken from an herbarium specimen (*R. Marloth 12398*, collected in 1925): “used by the Hottentots mixed with grease for the skin”.

In Smith (1966) it is claimed that the name of the San or Bushman people is derived from their use of aromatic shrubs to anoint their bodies (Van Wyk and Gericke, 2000). *Sab*, and also *San* or *Son*, is thought to be the original Nama (Khoi) names for *P. onobromoides*, from which the name *Sonqua* was derived. [This is one of several types of buchu, and the common name for this species in Afrikaans is *boegoebos* (“buchu bush”)]. *Sonqua* therefore literally means “bush people” or “bush men”, i.e. people (*qua*) who anoint their bodies with powdered aromatic bushes (*son*, *san*). As pointed out by Van Wyk (2008), the concept of *bossie* (Afrikaans for small bush) has special cultural and idiomatic significance in the semi-desert Karoo region of South Africa, where small shrubs are the dominant plant life form. The words *bos* or *bossie* in this context also mean “plant” or “herb”; for example, the traditional term (of honour) for a skilled traditional herbal doctor in this region is *bossiedokter*, i.e. a doctor who uses plants or herbs to heal (Van Wyk, 2008). *Sonqua* or *Tanqua* therefore appears to be the original Khoi/Nama names for the San people, translated into the Dutch *bosjesman*, which became *bossiesman* or *boesman* in Afrikaans and *bushman* in English.

There are no recent anecdotes about the traditional use of *P. onobromoides*. We were also unable to trace any local person in Namaqualand who still has original and detailed information about this species and its uses.

3.2. Anatomy

Oil glands appear macroscopically as translucent dots on the surface of the leaves (Fig. 1C). The leaves are amphistomatic and have a thin cuticle. The outer periclinal cell walls of the epidermal cells are highly cutinized. Palisade mesophyll occurs in both upper and lower parts of the leaf and consists of two or three layers of cells with occasional sinuate walls (Fig. 1D). The central spongy mesophyll cells have inconspicuous intercellular air spaces and walls frequently appearing sinuate. Secretory structures (Fig. 1E) are usually found below some of the vascular bundles in the spongy parenchyma adjacent to the palisade parenchyma. They are similar to those of the Rutaceae.

3.3. Essential oil composition

No published information about chemical compounds of any kind could be found for *P. onobromoides*. Considering the cultural and historical significance of the species, it is remarkable that it has remained unstudied for so long.

The essential oil yields of six individual plants collected from two different localities (Dwarskersbos and Lamberts Bay) are listed in Table 1. Yields were exceptionally variable, ranging from 0.0025% to 0.66% of dry weight. The variation seems to be unrelated to provenance and date of collection. The Lamberts Bay plants were in their pre-flowering (vegetative) state, while the Dwarskersbos plants were in full flower.



Fig. 2. Illustration of *Pteronia onobromoides* (“Carduus”) in Simon Van der Stel’s diary of the expedition to Namaqualand in 1685. The accompanying text (in Dutch) reads “this is a kind of Carduus and grows on the Dassenberg. Found on the 14th of September”.

A total of 78 volatile components were identified in the six samples studied. The major compounds are several monoterpenes as well as sesquiterpenes, listed in Table 2. Myrcene, limonene, 1,8-cineole and *p*-cymene are main compounds in practically all of the samples, with smaller amounts of sabinene, *trans*-linalooloxide, linalool, terpinen-4-ol, α -terpineol, eugenol and thymol. α -Phellandrene was present in three of the samples. A further 52 compounds were identified but these occur in trace amounts only and are not given in Table 2.

Despite considerable quantitative variation, the six samples, taken from three plants of two populations, were fairly uniform in having the combination of myrcene, limonene, 1,8-cineole and *p*-cymene as main constituents. The two populations are geographically widely separate, and the results indicate that this profile may be characteristic for *P. onobromoides*.

3.4. Antibacterial activity

The traditional topical use of *P. onobromoides* suggested that the plant may have some protective function on the skin, in addition to the more obvious value as a perfume. We therefore

examined, for the first time in this species, possible antibacterial activity using a selection of four bacteria that commonly infect the skin (Table 3).

The minimum inhibitory concentrations (MIC) of *P. onobromoides* were determined and the results (Table 3) showed that the methanol:dichloromethane (MeOH:CH₂Cl₂) extracts were moderately active against the two Gram-positive bacteria tested, *Staphylococcus aureus* and *Staphylococcus epidermidis*, as well as the two Gram-negative bacteria, *Pseudomonas aeruginosa* and *Proteus vulgaris*. The minimum inhibitory concentrations (Table 3) were as low as 1.00, 0.83, 2.00 and 1.00 mg/ml respectively.

The aqueous and 1:1 water: methanol extracts showed poor (5.33–8.00 mg/ml) or no activity against any of the tested organisms. The only exception was sample 7 (MeOH:H₂O extract) having moderate activity against *Staphylococcus epidermidis* (2.00 mg/ml). The essential oils also showed some activity (1.00–6.00 mg/ml) but were not as active as the MeOH:CH₂Cl₂ extracts. Extracts having MIC values below 8.00 mg/ml are considered to possess some antimicrobial activity (Fabry et al., 1998) and extracts having MIC values below 1.00 mg/ml are considered noteworthy (Gibbons, 2004; Rios and Recio, 2005). Essential oils having MIC values less than 2.00 mg/ml are considered noteworthy (Van Vuuren, 2008). The lowest minimum inhibitory concentration of 0.83 mg/ml (extract) for *Staphylococcus epidermidis* (sample 1) and 1.00 mg/ml (essential oil) for *Proteus vulgaris* (sample 4) is thus considered noteworthy and demonstrates that *P. onobromoides* was traditionally used for more than just cosmetic purposes. It is interesting to note that the traditional method of mixing powdered leaves with animal fat will be an efficient way of applying non-polar active compounds to the skin and that perhaps the essential oil alone does not account for the antibacterial activity of the plant. This twofold effect has recently been demonstrated in a study on *Tarchonanthus camphoratus*, where the non-volatile and volatile fractions in combination displayed an integral role in the total antimicrobial efficacy of the plant (Van Vuuren and Viljoen, 2009).

4. Conclusions

P. onobromoides is undoubtedly very important in the Nama cultural tradition, despite the paucity of recorded information about this aromatic plant and its uses. The earliest record dates from 1685, while the first record of topical medicinal application of powdered leaves mixed with fat was published in 1865. The traditional use as an important Nama “buchu” and the suggested origin of the names for Bushman people (*Sonqua*, *Sanqua* or *Tanqua*) is interesting and deserve further study. The anatomical studies showed that the essential oil is produced in globose oil vesicles situated below the vascular bundles of the leaf lamina. The essential oil is variable both in yield and in the levels of the main constituents but the combination of myrcene, limonene, 1,8-cineole and *p*-cymene appears to be characteristic for the species. Cosmetic and medicinal uses as a skin treatment and ointment are supported by the data. The pronounced antimicrobial activity of dichloromethane extracts against *Staphylococcus epidermidis* suggests that the plant may have been

used for its deodorant, protective and medicinal properties and not merely as a perfume.

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